

## Pharmacokinetics in Mice Implanted With Xenografted Tumors After Intravenous Administration of Tasidotin (ILX651) or Its Carboxylate Metabolite

Received: May 25, 2007; Final Revision Received: October 19, 2007; Accepted: October 19, 2007; Published: December 14, 2007

Peter L. Bonate,<sup>1</sup> David Beyerlein,<sup>2</sup> Jennifer Crawford,<sup>1</sup> Stephanie Roth,<sup>1</sup> Roy Krumbholz,<sup>1</sup> and Steve Schmid<sup>1</sup>

<sup>1</sup>Genzyme Corporation, 4545 Horizon Hill Blvd, San Antonio, TX 78229

<sup>2</sup>Microconstants, 9050 Camino Santa Fe, San Diego, CA 92121

### ABSTRACT

The pharmacokinetics of tasidotin (ILX651), a depsipeptide currently in phase II for the treatment of advanced solid tumors, and tasidotin-C-carboxylate, the main metabolite, were characterized in male nude mice implanted with LOX tumors, which are sensitive to tasidotin, or H460 tumors, which are resistant to tasidotin. The pharmacokinetics of tasidotin and its metabolites were characterized after single-dose administration of tasidotin (20 and 120 mg/kg), tasidotin-C-carboxylate (150 mg/kg), or tasidotin (53 mg/kg) in the presence and absence of Z-prolyl prolinal (5 mg/kg administered 1 hour prior to tasidotin administration), a competitive antagonist of prolyl oligopeptidase, the enzyme responsible for the metabolism of tasidotin to tasidotin-C-carboxylate. A secondary study was done comparing tumor growth in tasidotin-treated mice with implanted LOX tumors in the presence and absence of Z-prolyl-prolinal. After tasidotin administration, the pharmacokinetics of tasidotin and tasidotin-C-carboxylate were similar in plasma and tumors in LOX- and H460-implanted mice, indicating the resistance was not due to pharmacokinetic factors. Tumor carboxylate concentrations were much higher than in plasma after tasidotin administration. The metabolite appeared to contribute ~17% to 33% to the total exposure in LOX tumors and 20% to 49% in H460 tumors but <5% in plasma. Less than 5% of the administered tasidotin dose was converted to tasidotin-C-carboxylate, with no apparent differences between LOX- and H460-treated animals. The presence of Z-prolyl-prolinal decreased the amount of tasidotin converted to tasidotin-C-carboxylate from 5.5% to 0.90%, a reduction of almost 80%. After tasidotin-C-carboxylate administration, the half-life was on the order of minutes compared with hours when observed after tasidotin administration. Tasidotin-C-carboxylate elimination was not dependent on tasidotin pharmacokinetics, suggesting that the rate of efflux from cells into plasma was the rate-limiting step in its elimination. Tasidotin-C-carboxylate was also further metabolized to desprolyl-tasidotin-C-carboxylate,

although the metabolite ratios were <10%. Pretreatment with Z-prolyl-prolinal completely abolished the antitumor activity of tasidotin, indicating that the metabolite is the main moiety responsible for activity and that, despite tasidotin itself having activity in vitro, tasidotin is acting mainly as a prodrug.

**KEYWORDS:** Depsipeptide, solid tumors, metabolite kinetics, ILX651, prolyl oligopeptidase

### INTRODUCTION

The dolastatins are a group of structurally unique polypeptides containing unusual amino acids that were originally isolated from the Indian Ocean sea hare, *Dolabella auricularia*, and found to have significant preclinical antitumor activity.<sup>1</sup> Tasidotin (ILX651) is a third-generation synthetic dolastatin depsipeptide analog. Built using the cemadotin backbone,<sup>2</sup> tasidotin has a t-butylamide group instead of a benzylamide group at the C-terminus. The result is that tasidotin has greater metabolic stability than cemadotin in vitro. Mechanistically, tasidotin acts to inhibit cell proliferation by suppressing spindle microtubule dynamics through a reduction of the shortening rate, a reduction of the switching frequency from growth to shortening (catastrophe frequency), and a reduction of the time microtubules grow.<sup>3</sup>

Previous phase I studies in patients with advanced solid tumors refractory to prior therapies used 3 dosing regimens: once daily for 5 days every 3 weeks<sup>4</sup>; every other day for 5 days every 3 weeks<sup>5</sup>; and once weekly every 3 weeks.<sup>6</sup> Tasidotin showed promising activity with 1 complete response observed in the studies, that being a patient with advanced-stage metastatic melanoma, and several partial responses and incidences of stable disease. These studies indicated that tasidotin was rapidly cleared with a half-life of less than an hour across all doses examined. Furthermore, tasidotin was confined largely to the plasma compartment having a volume of distribution at steady state ranging from 8.1 to 13.2 L/m<sup>2</sup> with moderate between-subject variability of less than or equal to 44%. In all 3 phase I studies, mild nonlinearity in exposure was observed, with total systemic clearance decreasing with increasing dose. For a 2-fold increase

**Corresponding Author:** Peter L. Bonate, Genzyme Corporation, 4545 Horizon Hill Blvd, San Antonio, TX 78229. Tel: (210) 949-8662; Fax: (210) 949-8219; E-mail: [peter.bonate@genzyme.com](mailto:peter.bonate@genzyme.com)

in dose, a 2.3- to 2.4-fold increase in area under the curve from time 0 to infinity (AUC(0-∞)) was observed. Tasidotin renal clearance was minor, with ~10% to 14% of the dose excreted unchanged in urine.

Tasidotin was metabolized to tasidotin-C-carboxylate, also called the M1-metabolite, both in vitro and in vivo, as shown in Figure 1, through the loss of the N-t-butyl group at the C-terminus of the peptide forming the carboxylic acid. In the phase I studies, maximal plasma tasidotin-C-carboxylate concentrations were generally ~10% of maximal parent concentrations and, in contrast to parent exposure, tasidotin-C-carboxylate exposure was dose-proportional. Maximal tasidotin-C-carboxylate concentrations were reached within 5 to 8 hours after dosing. Tasidotin-C-carboxylate half-life, estimated to be ~8 hours, was much longer than parent half-life. Because of the longer half-life, the metabolite ratio was estimated to be 0.59, indicating that tasidotin-C-carboxylate contributed ~59% to the total exposure.<sup>4-6</sup>

In A549 cells incubated with tasidotin, tasidotin was rapidly taken up into cells where it was metabolized to the more active metabolite tasidotin-C-carboxylate by cleavage of the t-butyl-amine group via the enzyme prolyl oligopeptidase (POP).<sup>7</sup> Tasidotin-C-carboxylate was then metabolized to desprolyl-tasidotin-C-carboxylate, also called the M2-metabolite, releasing free proline by an unknown enzyme. Both analytes were substrates for p-glycoprotein, but tasidotin appeared to be the stronger substrate. Uptake of

tasidotin-C-carboxylate from the extracellular medium also occurred, but the exact uptake mechanism remains unclear. Although the absolute amount of radioactivity taken up into cells was only a few percentage points for both tasidotin and tasidotin-C-carboxylate, both showed ~30% to 40% retention after 24 hours.

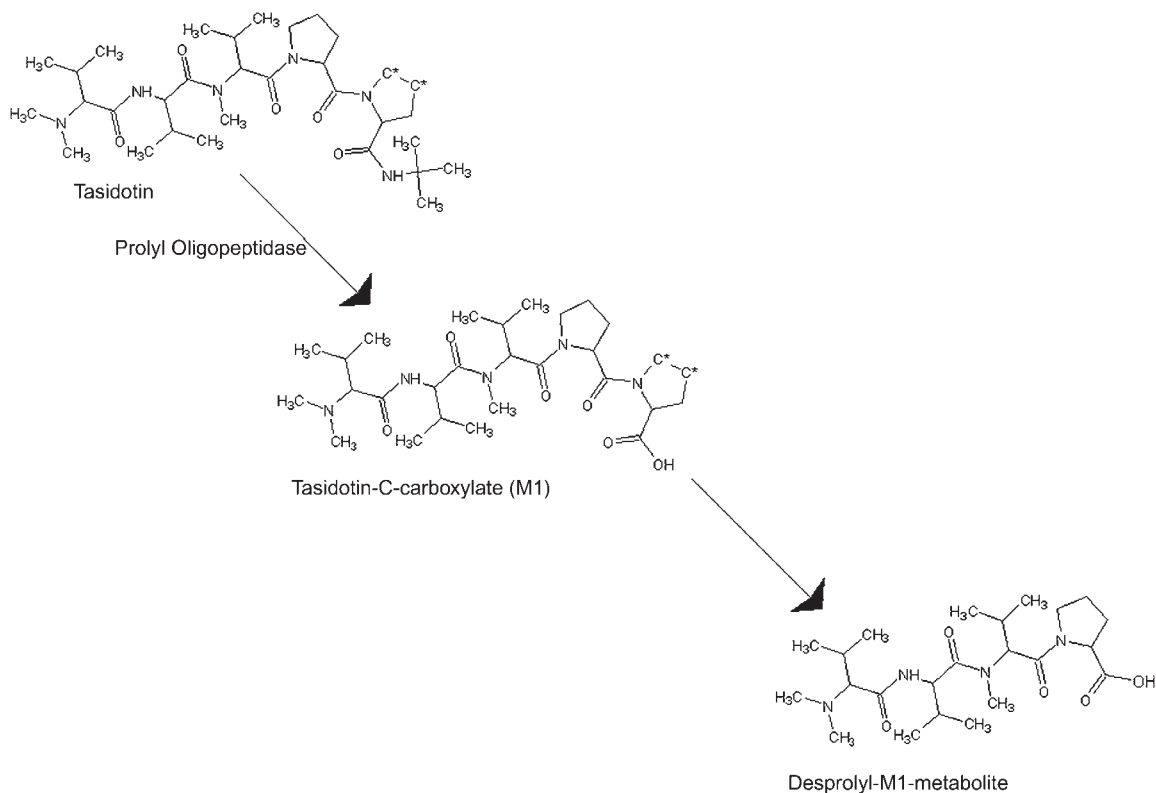
The purpose of this study was to characterize the pharmacokinetics of tasidotin, tasidotin-C-carboxylate, and desprolyl-tasidotin-C-carboxylate in plasma and tumors after single-dose intravenous administration in male nude mice implanted with xenograft tumors and to further understand the relationship of tasidotin-C-carboxylate to pharmacodynamic efficacy.

## METHODS

### Pharmacokinetic Studies

#### Single-Dose Intravenous Administration of Tasidotin in Xenograft Mice

Male (nu/nu) mice 6 to 7 weeks of age (Harlan Sprague Dawley, Indianapolis, IN) weighing ~25 g were used. Mice were implanted subcutaneously in the axillary region by trocar with fragments of LOX human melanoma carcinomas or H460 non-small cell lung tumors harvested from subcutaneously grown tumors in nude mice hosts. When the tumors were ~330 mm<sup>3</sup> in size (8 days following



**Figure 1.** Metabolism of tasidotin.

implantation), the animals were pair-matched into treatment and control groups. Each time point contained 3 mice bearing tumors, each of which was ear-tagged and followed individually throughout the experiment. Tumors were measured the day of pair-matching. These tumor measurements were converted to mm<sup>3</sup> tumor volume by the following formula: {width (mm)<sup>2</sup> × length (mm)} × 0.52.

Test article administration began 1 day after pair-matching. Tasidotin was administered as a single dose intravenously via the tail vein at 20 or 120 mg/kg. Tasidotin was formulated in saline and administered to all of the groups. Baseline samples were included in each study. Treatment and baseline groups were dosed at a constant volume of 10 mL/kg. The baseline group did not receive any treatment, and the blood and tumor tissues from these animals served as negative controls.

After dosing and at the various time points, the mice were anesthetized with halothane and 200 to 400 µL of whole blood was collected per mouse via cardiac puncture. In the mice implanted with LOX tumors, samples were collected at 0, 5, 15, 30, 60, 90, 120, 150, 240, and 480 minutes after dosing for both dosing groups. In mice implanted with H460 tumors, samples were collected at 0, 5, 15, 30, 60, 90, 120, 150, 240, and 480 minutes in the 20 mg/kg dose group and at 0, 5, 15, 30, 60, 120, 180, 240, 480, and 1440 minutes in the 120 mg/kg dose group. The whole blood was placed in lithium heparin Microtainer tubes and centrifuged at 9000 rpm for 10 minutes. Individual mouse plasma was then collected and placed in Eppendorf tubes and flash frozen. Once the blood was collected from each mouse, the tumors were excised, weighed, placed in cryovials, and flash frozen. All of the samples were stored in the -70°C freezer until shipment. Plasma and tumor samples were analyzed for tasidotin and tasidotin-C-carboxylate.

#### *Single-Dose Intravenous Administration of Tasidotin-C-Carboxylate*

The same methods were used for administration of 150 mg/kg tasidotin-C-carboxylate as were used for administration of tasidotin. Mice were implanted with LOX carcinomas. Plasma and tumor samples were collected at baseline and 5, 15, 30, and 60 minutes, and 2, 3, 4, 8, 24, and 48 hours postdose. All samples were analyzed for tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate by liquid chromatography with tandem mass spectral detection (LC-MS/MS).

#### *Single-Dose Intravenous Administration of Tasidotin in the Presence and Absence of Z-Prolyl Prolinal, a Competitive Inhibitor of POP*

The pharmacokinetics of tasidotin and tasidotin-C-carboxylate were characterized in the presence and absence of Z-prolyl

prolinal, a competitive inhibitor of POP. In the control group, male nu/nu mice (not with tumors) were administered 53 mg/kg tasidotin intravenously. In the active treatment group, male nu/nu mice were administered 5 mg/kg Z-prolyl prolinal 1 hour prior to administration of 53 mg/kg tasidotin. Serial blood samples were collected for 48 hours with 3 animals per time point. The plasma concentrations of tasidotin and tasidotin-C-carboxylate were analyzed by LC-MS/MS.

#### *Bioanalytical Methods*

Plasma and tumor samples were analyzed for tasidotin, tasidotin-C-carboxylate, and desprolyl-tasidotin-C-carboxylate at MicroConstants, Inc (San Diego, CA) using high-performance liquid chromatography (HPLC) with mass spectrometric (MS/MS) detection. Mouse tumors were weighed, and then 50 mM potassium phosphate was added to the tube for a final concentration of 100 mg/mL. The tumors were subsequently homogenized using a Polytron 1200C (Brinkmann, Westbury, NY). Tumor homogenate and plasma samples were spiked with β-casomorphin (1-4) amide as the internal standard. The samples were precipitated with acetonitrile and the organic layer was evaporated, then reconstituted in water:acetonitrile (80:20:0.1, vol/vol).

The samples after tasidotin administration were analyzed by reversed-phase liquid chromatography using an XDB-phenyl column maintained at 45°C. The mobile phase was nebulized using heated nitrogen in a Z-spray source/interface, and the ionized compounds were detected using a tandem quadrupole mass spectrometer (Quattro II, Waters Micromass, Milford, MA). The HPLC mobile phase was a gradient consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The column was an XDB-phenyl, 150 × 2.1 mm, 5 µm (Agilent Technologies, Santa Clara, CA) column with a flow rate of 0.300 mL/min maintained at 45°C. Column switching was 0.1 to 4.0 minutes. Sample temperatures were ambient. The total analysis time was ~9.0 minutes. The injection volume was 20.0 µL. Under these conditions the column back pressure was 90 ± 40 bar with the following retention times: tasidotin, 4.7 ± 0.3 minutes; tasidotin-C-carboxylate, 4.4 ± 0.3 minutes; and internal standard, 4.4 ± 0.3 minutes. Mass spectrometer conditions were as follows: source temperature: 130°C; desolvation temperature: 350°C; nebulizing gas (nitrogen): ~100 L/hr; drying gas (nitrogen): ~650 L/hr. Tasidotin had a cone of 25 V, a collision energy of 19 eV, and mass transitions monitored from 607.65 → 340.30. Tasidotin-C-carboxylate had a cone of 25 V, a collision energy of 19 eV, and mass transitions monitored at 552.50 → 340.30. The internal standard had a cone of 40 V, a collision energy of 18 eV, and mass transitions monitored from 522.40 → 408.20. The linear range of the method was 5.00

to 1000.0 ng/mL for tasidotin and 1.00 to 1000 ng/mL for tasidotin using 50  $\mu$ L of mouse plasma or tumor homogenate. The bias of the method based on the examined standards did not exceed  $\pm 10\%$  for any analyte.

The samples after tasidotin-C-carboxylate administration were analyzed using the same method as after tasidotin administration with the following exceptions: column temperature of 40°C and column switching of 0.1 to 2.8 minutes. Under these conditions the column back pressure was  $90 \pm 40$  bar with the following retention times: tasidotin-C-carboxylate,  $3.4 \pm 0.2$  minutes; desprolyl-tasidotin-C-carboxylate,  $3.4 \pm 0.2$  minutes; and internal standard,  $3.4 \pm 0.2$  minutes. Mass spectrometer conditions were the same as for the tasidotin assay with the following exceptions: nebulizing gas (nitrogen),  $\sim 20$  L/hr; and drying gas (nitrogen),  $\sim 800$  L/hr. Tasidotin-C-carboxylate had a cone of 19 V, a collision energy of 18 eV, and mass transitions monitored from 552.25  $\rightarrow$  340.25. Desprolyl-tasidotin-C-carboxylate had a cone of 17 V, a collision energy of 15 eV, and mass transitions monitored from 455.20  $\rightarrow$  340.25. The internal standard had a cone of 29 V, a collision energy of 19 eV, and mass transitions monitored from 522.15  $\rightarrow$  408.00. The linear range of the method was 1.00 to 500.0 ng/mL for all analytes using 50.0  $\mu$ L of mouse plasma or tumor homogenate. The bias of the method based on the examined standards did not exceed  $\pm 10\%$  for any analyte.

#### Analysis Methods

The data set was examined for potential outliers prior to analysis. No formal statistical analysis was done to confirm any identified outliers. Outliers were identified if the observed concentration was more than 5-fold higher than the next highest concentration in the dose cohort at the same time point and matrix. In the LOX-implanted mice after administration of tasidotin, the tasidotin concentration in plasma at 120 minutes was removed from the analysis as an outlier (23327 ng/mL vs 2992 and 5870 ng/mL), as was the tasidotin-C-carboxylate concentration in plasma at 480 minutes postdose (99.5 ng/mL vs 1.2 and 1.8 ng/mL). In the H460-implanted mice after administration of tasidotin, 1 tasidotin concentration outlier at 480 minutes postdose was removed from the tumor samples in the 20 mg/kg dose group (303 ng/mL vs 2.48 and 7.98 ng/mL). In that same dose group and matrix at 180 minutes postdose, another sample was removed as an outlier (159 ng/mL vs 20.6 and 18.7 ng/mL). In the 20 mg/kg dose group at 480 minutes postdose, 1 tasidotin plasma concentration was removed as an outlier (12.3 ng/mL vs below the quantification limit [BQL]). One sample following tasidotin-C-carboxylate administration was removed as a pharmacokinetic outlier. The plasma sample at 30 minutes postdose had a concentration of 112 373 ng/mL compared with concentrations of 1734 and 1844 ng/mL in the remaining 2 mice in that cohort.

Plasma and tumor concentrations were analyzed using non-compartmental methods with WinNonlin Professional (Version 5, Pharsight Corp, Mountain View, CA). Samples BQL prior to the maximal concentration were set equal to 0, whereas samples that were BQL in the terminal phase were set to missing. AUC(0-last) was estimated using the linear trapezoidal rule from time 0 to the last measurable concentration. Maximal concentrations ( $C_{max}$ ) and time to maximal concentration ( $T_{max}$ ) were estimated from direct examination of the data. Terminal and effective half-life<sup>8</sup> were estimated using log-linear regression of at least 3 concentration-time points in the particular phase of the concentration-time profile where the coefficient of determination was at least 0.90. Both effective half-life and terminal half-life were estimated whenever possible because of the multiphasic character of their disposition profiles. AUC from time 0 extrapolated to  $\infty$  was calculated as the sum of AUC(0-last) plus the last measurable concentration divided by the terminal elimination rate constant. Metabolite ratios corrected for molecular weight were calculated using Equation 1:

Metabolite Ratio =

$$\frac{AUC(0-\infty)_{metabolite} \left( \frac{\text{nmol}}{551.7 \text{ Da}} \right) \times 100\%}{AUC(0-\infty)_{parent} \left( \frac{\text{nmol}}{606.8 \text{ Da}} \right) + AUC(0-\infty)_{metabolite} \left( \frac{\text{nmol}}{551.7 \text{ Da}} \right)} \quad (1)$$

Tumor-to-plasma ratios were calculated as follows:

$$\text{Tumor-to-Plasma Ratio} = \frac{AUC(0-\infty)_{tumor}}{AUC(0-\infty)_{plasma}} \quad (2)$$

In the case following tasidotin-C-carboxylate administration, AUC(0- $\infty$ ) was replaced by AUC(0-last).

The fraction of parent drug metabolized to tasidotin-C-carboxylate was calculated as follows:

$$f_m = \frac{CL_m \times AUC_m \times MW_p}{Dose_p \times MW_m} \quad (3)$$

where  $CL_m$  is the metabolite clearance after administration of the metabolite,  $AUC_m$  is the area under the curve for the metabolite after administration of the parent drug,  $MW_p$  is the molecular weight of tasidotin (606.8 Da),  $Dose_p$  is the administered dose of the parent drug, and  $MW_m$  is the molecular weight of tasidotin-C-carboxylate (551.7 Da).

#### ***Xenograft Study With Tasidotin and Tasidotin-C-Carboxylate in the Presence and Absence of Z-Prolyl-Prolinal, a Competitive Inhibitor of POP***

Male nude (nu/nu) mice 6 weeks of age (Harlan Sprague Dawley, Indianapolis, IN) weighing  $\sim 20$  to 24 g at the time of tumor implantation were used. Mice were implanted subcutaneously in the axilla region by trocar with fragments of LOX human melanoma tumors harvested from

subcutaneously growing tumors in nude mice hosts. When the tumors were ~176 to 186 mm<sup>3</sup> in size (7 days following implantation), the animals were pair-matched into treatment and control groups. Each group contained 9 mice bearing tumors, each of which was ear-tagged and followed individually throughout the experiment.

A stock tasidotin solution of 5.3 mg/mL was prepared by adding the appropriately weighed amount of test article to the appropriate volume of saline to achieve a clear solution. This stock solution was then diluted to the lower concentrations through serial dilutions. Dosing mixtures were vortexed for ~10 seconds until the test article was dissolved. Dosing solutions were prepared daily in sterile 15-mL polypropylene centrifuge tubes.

A stock solution of Z-prolyl-prolinal, a competitive inhibitor of POP, of 0.5 mg/mL was prepared by adding the appropriate weighed amount of test article to 10% methanol to achieve a clear solution, then diluted to the appropriate volume with water for injection. Dosing solutions were prepared immediately prior to dosing in sterile scintillation vials.

Prepared test article dosing solutions used on the day of preparation were maintained at controlled ambient temperature and during dosing and sampling. Prepared test article dosing solutions not used on the day of preparation were discarded. The administration of vehicle or test agents began the same day as pair-matching. The doses were administered orally or interperitoneally at a constant dose volume of 10 mL/kg based upon each animal's body weight at that time.

Six groups of animals were studied:

- Untreated control group
- Control group with saline (tasidotin vehicle) administered orally every other day for 3 days for 3 cycles
- Control group with Z-prolyl-prolinal (10% methanol in water) administered intraperitoneally once daily for 5 days
- Group with tasidotin 53 mg/kg administered once daily every other day for 3 days every week for 3 cycles (Because of the rapid growth of the tumor, only 1 cycle of tasidotin was administered before study termination.)
- Group with Z-prolyl-prolinal 5 mg/kg administered once daily for 5 days
- Group with tasidotin 53 mg/kg administered once daily every other day for 3 days every week for 3 cycles, and Z-prolyl-prolinal 5 mg/kg administered once daily for 5 days, 30 minutes prior to tasidotin administration

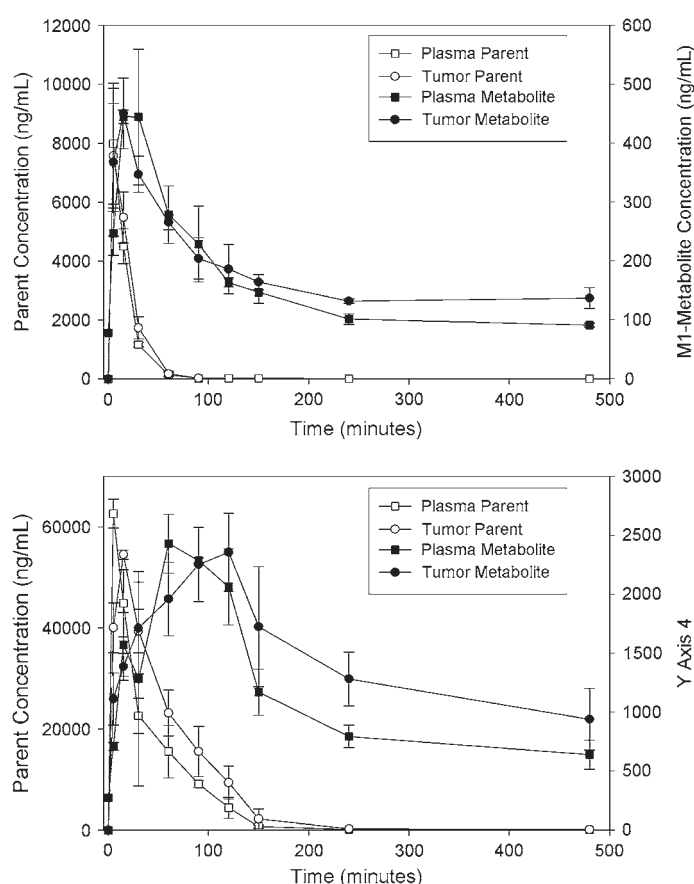
The dose of tasidotin chosen for the study was based on prior studies with this xenograft model (Schmid S., unpublished data, January 2004), where the dose had been shown

to be efficacious. The dose of Z-prolyl prolinal was chosen based on a literature review showing that at 5 mg/kg, POP activity was significantly reduced<sup>7</sup> and decreased metabolite formation was observed in vivo for a prototypical POP substrate, Z-Gly-Pro-sulfamethoxazole.<sup>9</sup> Tumor volumes were monitored twice weekly as described in earlier sections. All mice were individually weighed prior to each dose, but weights were recorded only twice weekly. Abnormal clinical signs were recorded for all mice before each dosing and frequently after each dose. Abnormal clinical signs were recorded on all mice at the time of body weight measurement on nondosing days. Mortality evaluations were performed on all mice daily.

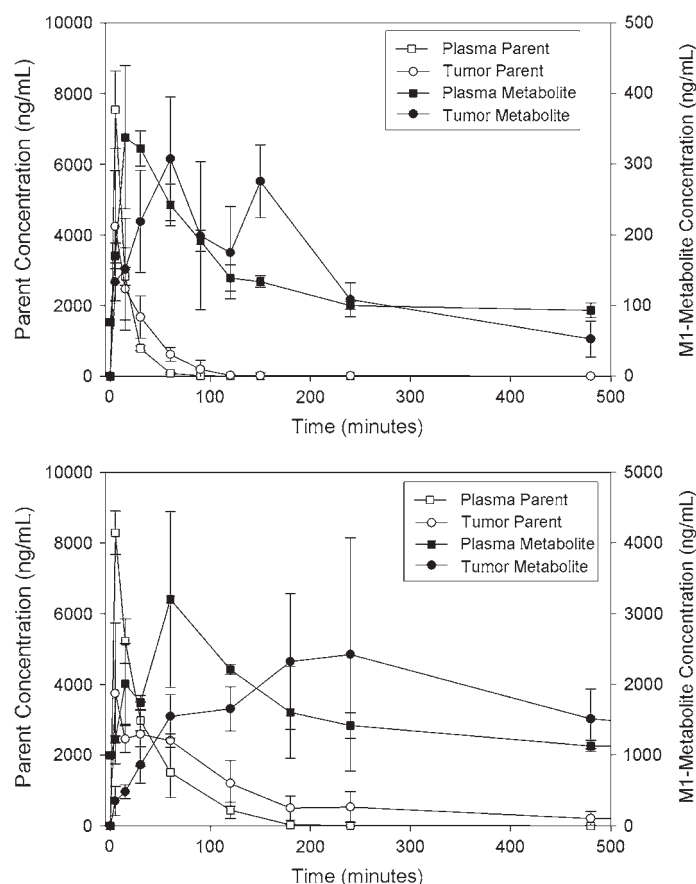
## RESULTS

### Single-Dose Intravenous Administration of Tasidotin

Figures 2 and 3 present scatter plots of mean tasidotin and tasidotin-C-carboxylate concentrations over time after intravenous administration of 20 or 120 mg/kg in mice implanted with LOX or H460 tumors. Table 1 presents a summary of the pharmacokinetics of tasidotin and tasidotin-C-carboxylate in plasma and tumors.



**Figure 2.** Mean parent and metabolite concentrations in plasma and tumor after administration of 20 mg/kg (top) or 120 mg/kg (bottom) tasidotin in mice implanted with LOX tumors. Error bars are SD. Note the different scale for metabolite concentrations.



**Figure 3.** Mean parent and metabolite concentrations in plasma and tumor after administration of 20 mg/kg (top) or 120 mg/kg (bottom) tasidotin in mice implanted with H460 tumors. Error bars are SD. Note the different scale for metabolite concentrations.

The ratio of matrix-, dose-, analyte-, and time-matched H460 to LOX concentrations was calculated. Most values were around 1.0, but 3 values were considered outliers. After removal of the outliers, no dramatic differences were observed in the ratios between the H460 and LOX treatment groups. Most ratios tended to center around 1.0, which would indicate that the concentrations were equal between the treatment groups.

Tasidotin did not show linear plasma pharmacokinetics, as exposure was supraproportional at 120 mg/kg compared with 20 mg/kg. When the dose was increased 6-fold from 20 to 120 mg/kg, plasma AUC(0-∞) increased 16-fold in LOX-implanted mice and 26-fold in H460-implanted mice. Tumor AUC(0-∞) increased 21-fold in LOX-implanted mice and 41-fold in H460-implanted mice. In contrast, maximal concentrations appeared to be proportional or at least not as non-linear as AUC. A 6-fold increase in dose resulted in an 8-fold increase in LOX-implanted mice and an 11-fold increase in H460-implanted mice. Hence, the nonlinearity was expressed as a supraproportional increase in AUC(0-∞) with less non-linear increase in C<sub>max</sub>. Also noteworthy was that tasidotin tumor concentrations were similar to tasidotin plasma concentrations, as their concentration-time profiles were virtually

superimposable and tumor-to-plasma ratios were 1.1 to 1.8, with little tasidotin retention seen in tumors.

Tasidotin-C-carboxylate formed slowly compared with the elimination kinetics of tasidotin, with metabolite plasma and tumor concentrations being a small fraction of parent concentrations in their respective matrices. T<sub>max</sub> for the metabolite was 15 to 240 minutes depending on the treatment group, dose, and matrix. In contrast to tasidotin, tasidotin-C-carboxylate appeared to show linear pharmacokinetics in both plasma and tumors. Plasma AUC(0-last) increased 6-fold in LOX-implanted mice and 11-fold in H460-implanted mice. Tumor AUC(0-last) increased 8-fold in LOX-implanted mice and 27-fold in H460-implanted mice. Plasma C<sub>max</sub> increased 3-fold in LOX-implanted mice and 7-fold in H460-implanted mice. Tumor C<sub>max</sub> increased 7-fold in LOX-implanted mice and 8-fold in H460-implanted mice.

Tasidotin-C-carboxylate's terminal half-life was significantly longer than tasidotin's, although an accurate assessment of metabolite half-life could not be made because of the sampling scheme. As a rough estimate, tumor tasidotin-C-carboxylate's terminal half-life was 6 to 7 hours, compared with an effective half-life of less than 30 minutes and a terminal half-life of 1 to 4 hours for tasidotin.

Tasidotin-C-carboxylate concentrations were much higher in tumors than in plasma, with tumor-to-plasma ratios of 19.9 to 48.6. Although tumor and plasma concentrations of metabolite were substantially less than concentrations of parent (<10%), metabolite concentrations constituted a meaningful proportion of total tumor exposure because of the metabolite's longer elimination half-life. Based on the metabolite ratios, the metabolite appeared to contribute ~17% to 33% to the total exposure in LOX tumors and 20% to 49% to the total exposure in H460 tumors.

### Single-Dose Intravenous Administration of Tasidotin-C-Carboxylate

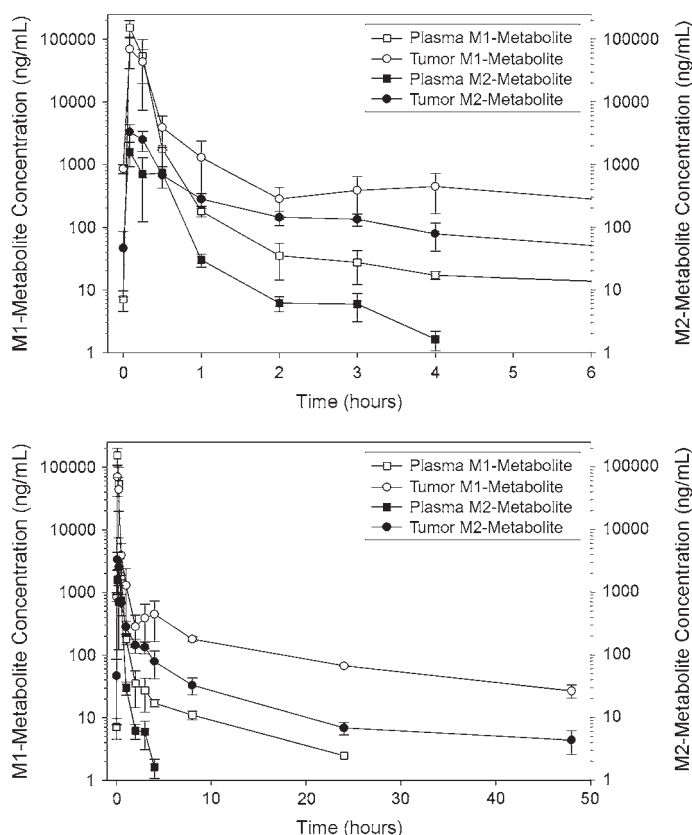
Figure 4 presents scatter plots of mean tasidotin-C-carboxylate and despropyl-tasidotin-C-carboxylate concentrations over time after intravenous administration of 150 mg/kg, in mice implanted with LOX tumors. Table 2 presents a summary of the pharmacokinetics of tasidotin-C-carboxylate and despropyl-tasidotin-C-carboxylate in plasma and tumors. Tasidotin-C-carboxylate concentrations declined multiphasically with a rapid effective half-life on the order of minutes in both plasma and tumors. Within 1 hour after administration, concentrations declined to <10% of maximal concentrations. Initially concentrations were higher in plasma, but at later time points tumor concentrations were higher than plasma concentrations. Tumor tasidotin-C-carboxylate concentrations remained persistent for a long period of time, having a terminal half-life of 48 hours, with concentrations being

**Table 1.** Summary of Pharmacokinetics of Tasidotin and Tasidotin-C-Carboxylate in Plasma and Tumors in Mice Implanted with LOX or H460 Tumors\*

LOX Tumor Type	Tasidotin			
	Plasma		Tumor	
	20 mg/kg	120 mg/kg	20 mg/kg	120 mg/kg
AUC(0-last) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	151	2480	170	3492
AUC(0- $\infty$ ) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	154	2489	170	3512
AUC(0- $\infty$ )/dose	7689	20 739	8501	29 266
Cmax (ng/mL)	7980	62 631	7568	54 503
Clearance (mL/min/kg)	130	48	118	34
Vdss (mL/kg)	5361	2209	1974	1982
Effective half-life (min)	10	21	10	28
Terminal half-life (min)†	339	155	Missing	162
Tumor-to-plasma ratio	—	—	1.1	1.4
H460 Tumor Type	Tasidotin-C-Carboxylate			
	Plasma		Tumor	
	20 mg/kg	120 mg/kg	20 mg/kg	120 mg/kg
Cmax (ng/mL)	42.2	142	450	2356
Tmax (min)	15	60	15	120
AUC(0-last) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	4.2	26.7	83.9	689
AUC(0-last)/dose	211	223	4196	5745
Terminal half-life (min)†	283	484	Missing	402
Metabolite ratio (%)	3.0	1.2	35.2	17.8
Tumor-to-plasma ratio	—	—	19.9	25.8
H460 Tumor Type	Tasidotin			
	Plasma		Tumor	
	20 mg/kg	120 mg/kg	20 mg/kg	120 mg/kg
AUC(0-last) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	113	2936	129	5369
AUC(0- $\infty$ ) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	113	2937	130	5377
AUC(0- $\infty$ )/dose	5633	24 475	6509	44 811
Cmax (ng/mL)	7541	82 901	4235	37 480
Clearance (mL/min/kg)	178	41	154	22
Vdss (mL/kg)	2467	1708	6376	4443
Effective half-life (min)	10	20	16	53
Terminal half-life (min)†	30	91	182	166
Tumor-to-plasma ratio	—	—	1.2	1.8
H460 Tumor Type	Tasidotin-C-Carboxylate			
	Plasma		Tumor	
	20 mg/kg	120 mg/kg	20 mg/kg	120 mg/kg
Cmax (ng/mL)	31	220	308	2426
Tmax (min)	15	60	60	240
AUC(0-last) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	3.45	38.4	68.9	1869
AUC(0-last)/dose	172	320	3447	15 573
Terminal half-life (min)†	190	222	150	580
Metabolite ratio (%)	3.3	1.4	37.0	27.7
Tumor-to-plasma ratio	—	—	20.0	48.6

\*AUC indicates area under the curve.

†A rough estimate. “Missing” indicates that the value could not be determined because there was an insufficient number of samples to accurately estimate the value.



**Figure 4.** Scatter plot of mean M1- and M2-metabolite concentrations in plasma and tumor after administration of 150 mg/kg M1-metabolite in mice implanted with LOX tumors. Error bars are SD.

~1% of maximal concentrations. The tumor-to-plasma ratio for tasidotin-C-carboxylate was 0.83, which indicated that plasma tasidotin-C-carboxylate was about in equilibrium with tumor tasidotin-C-carboxylate.

Little despropyl-tasidotin-C-carboxylate was formed following tasidotin-C-carboxylate administration. Maximal concentrations were ~1% of tasidotin-C-carboxylate concentrations, and the metabolite ratio was ~2.5% in plasma and 10.2% in tumors. Despropyl-tasidotin-C-carboxylate was cleared more rapidly than tasidotin-C-carboxylate, as the former had a shorter half-life than the latter.

After administration of 20 or 120 mg/kg tasidotin, and using the estimate of metabolite clearance following administration of the metabolite, the fraction of tasidotin converted to tasidotin-C-carboxylate was 1.8% and 6.1% in LOX-implanted animals, respectively, and 1.5% and 5.0% in H460-implanted animals.

#### **Single-Dose Intravenous Administration of Tasidotin in the Presence and Absence of Z-Propyl Prolinal, a Competitive Inhibitor of POP**

The total AUC for tasidotin and tasidotin-C-carboxylate in the control group was 630 and 33.4  $\mu\text{g}\cdot\text{h}/\text{mL}$ , respectively.

**Table 2.** Summary of Pharmacokinetics of Tasidotin-C-Carboxylate and Despropyl-Tasidotin-C-Carboxylate in Plasma and Tumors in Mice Implanted with LOX Tumors After Intravenous Administration of 150 mg/kg Tasidotin-C-Carboxylate\*

Parameter	Tasidotin-C-Carboxylate	
	Plasma	Tumor
AUC(0-last) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	1885	1533
AUC(0- $\infty$ ) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	1886	1567
Cmax ( $\mu\text{g}/\text{mL}$ )	154	69.8
Clearance ( $\text{mL}/\text{min}/\text{kg}$ )	79.5	95.7
Vdss ( $\text{mL}/\text{kg}$ )	1127	24 762
Effective half-life (min)	5.8	9.6
Terminal half-life (min)	1440	2880
Tumor-to-plasma ratio	—	0.83
Parameter	Despropyl-Tasidotin-C-Carboxylate	
	Plasma	Tumor
Cmax ( $\text{ng}/\text{mL}$ )	1585	3322
Tmax (min)	5	5
AUC(0-last) ( $\mu\text{g}\cdot\text{min}/\text{mL}$ )	39.3	144
AUC(0- $\infty$ )	39.4	146
Effective half-life (min)	Missing	15.1
Terminal half-life (min)	240	1440
Metabolite ratio (%)	2.5	10.2
Tumor-to-plasma ratio	—	3.70

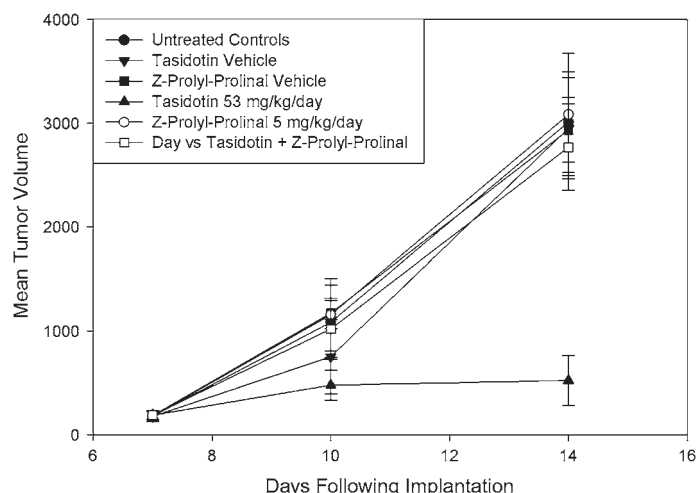
\*AUC indicates area under the curve. “Missing” denotes that the value could not be determined.

The total AUC for tasidotin and tasidotin-C-carboxylate following pretreatment with Z-propyl-prolinal was 532 and 5.4  $\mu\text{g}\cdot\text{h}/\text{mL}$ , respectively. The metabolite ratio in the control group was 5.5% and 1.1% in the presence of Z-propyl-prolinal, a reduction of almost 80%. The fraction of metabolite formed in the control group was 5.5% and 0.9% in the presence of Z-propyl-prolinal, a reduction of almost 84%.

#### **Xenograft Study With Tasidotin and Tasidotin-C-Carboxylate in the Presence and Absence of Z-Propyl-Prolinal, a Competitive Inhibitor of POP**

Figure 5 presents a scatter plot of mean tumor volumes after administration of tasidotin in the presence and absence of Z-propyl-prolinal. Tasidotin produced low tumor growth inhibition activity at the dosage tested. This study was terminated before the animals in this group reached the study end point of 2000  $\text{mm}^3$ , so a delay in tumor growth could not be determined. It should be noted that at the time of study termination, the mean tumor volume of the group was 520.2  $\text{mm}^3$ .

Z-propyl-prolinal did not exhibit antitumor activity against this tumor line. All of the animals reached the study end point



**Figure 5.** Scatter plot of mean tumor volume in mice implanted with LOX tumors following treatment with tasidotin in the presence and absence of Z-prolyl-prolinal. Error bars are SD.

by day 14. Tasidotin in combination with Z-prolyl-prolinal was not efficacious. As with the single-agent Z-prolyl-prolinal group, all of the mice reached 2000 mm<sup>3</sup> by day 14. All of the groups' treatments were well tolerated, producing <20% weight loss and no toxic deaths. It was concluded that Z-prolyl-prolinal abolished the activity of tasidotin.

## DISCUSSION

This study revealed no dramatic differences in the tasidotin and tasidotin-C-carboxylate pharmacokinetics between mice implanted with H460 (a type of lung tumor) and LOX (a type of melanoma tumor). The general shape and magnitude of the concentration-time profiles for both analytes were similar in both tumor and plasma at the 2 doses examined. These data suggest that the mechanism underlying H460 resistance to tasidotin was not inadequate tasidotin exposure. The mouse xenograft study with Z-prolyl-prolinal also clearly showed that formation of tasidotin-C-carboxylate was required for activity. When a competitive inhibitor of POP, the main enzyme responsible for the formation of tasidotin-C-carboxylate, was administered prior to tasidotin, the antitumor effects of tasidotin were completely abolished. Hence, although tasidotin had cytotoxic effects *in vitro*, *in vivo* it would appear to act as more of a prodrug.

This study also characterized the pharmacokinetics of tasidotin and its metabolites, tasidotin-C-carboxylate and desprolyl-tasidotin-C-carboxylate, after single-dose administration of tasidotin or tasidotin-C-carboxylate. Tasidotin itself was rapidly cleared from the plasma with an effective half-life of less than 30 minutes. Tasidotin penetration was adequate and plasma concentrations were in rapid equilibrium with tumors. It should be noted, however, that the tumor concentrations measured in this study represent a combination of intracellular,

extracellular, and residual blood. Thus, while the intracellular concentration, which is of primary interest, was not being measured, the results show that there is good potential for tasidotin to have high intracellular concentrations.

Tasidotin-C-carboxylate, the main metabolite of tasidotin, appeared to be slowly eliminated and did not contribute to exposure to any significant extent in plasma after tasidotin administration. In tumors, metabolite concentrations were less than parent concentrations, but the metabolite was retained in the cell for a much longer period of time. Hence, in tumors, while total exposure was due predominantly to tasidotin, anywhere from 20% to 50% appeared to be due to tasidotin-C-carboxylate.

Intravenous administration of tasidotin showed that tasidotin-C-carboxylate had an apparent effective half-life of at least 4 hours. Following tasidotin-C-carboxylate administration, tasidotin-C-carboxylate was rapidly cleared from the body with an effective half-life on the order of minutes. Metabolite kinetics are usually classified as either formation rate-limited or not formation rate-limited. Tasidotin-C-carboxylate elimination was neither. Formation rate-limited elimination occurs when the apparent half-life of the metabolite is the same as the half-life of the parent after administration of the parent, but in fact the elimination of the metabolite is faster than the elimination of the parent. That was not the case herein. After administration of tasidotin, tasidotin-C-carboxylate half-life was much longer than the parent half-life. This would suggest that metabolite elimination was not formation rate-limited and that the rate of elimination of the metabolite was slower than the parent's. However, in this study metabolite elimination after administration of the metabolite was considerably faster than metabolite elimination after administration of the parent, the opposite of what would be expected. This conflicting result can be rationalized by the hypothesis that the rate-limiting step in the clearance of the metabolite is not metabolism but the rate of efflux from the cells, which is where the metabolite is formed, into plasma. If efflux is slower than metabolism, the half-life will appear longer after administration of the parent.

Tasidotin-C-carboxylate also appeared to show good penetration into the tumor after intravenous administration, with tumor concentrations being almost equal to plasma concentrations, at least initially. At later time points, tumor tasidotin-C-carboxylate concentrations were higher than in plasma. However, as has already been discussed, tumor concentrations in this instance represent both intracellular and extracellular concentrations, including residual blood in the tumor. Since tasidotin-C-carboxylate is a carboxylic acid, it is not expected to penetrate tumors to any extent by diffusion. It is difficult to ascertain whether tumor concentrations reflect intracellular tasidotin-C-carboxylate or intracellular plus extracellular concentrations. *In vitro*

studies with A549 cells indicated that following incubation with tasidotin-C-carboxylate, tasidotin-C-carboxylate was indeed identified inside the cell, but the relative amount of intracellular concentration to extracellular concentration was just a few percent (Au J., written communication, January 2007). Hence, it would appear that in this study the ratio of tasidotin-C-carboxylate in tumors to tasidotin-C-carboxylate in plasma was probably an artifact.

The formation of tasidotin-C-carboxylate has been previously shown to be catalyzed by the enzyme POP (Jurutka P., personal communication, December 2005), also called vascular post-proline cleaving enzyme and proline endopeptidase.<sup>10,11</sup> This enzyme is a ubiquitous cytosolic serine protease that metabolizes several active peptides, including angiotensin II, vasopressin, and Substance P. Little POP activity is observed in the plasma compared with in the tissues, and the source of plasma enzyme remains controversial, although the dominant theory is leakage of intracellular enzyme following natural cell death. It would appear that tasidotin is taken up into cells where it is metabolized to tasidotin-C-carboxylate. Tasidotin-C-carboxylate is then effluxed from the cell, is metabolized to desprolyl-tasidotin-C-carboxylate, or diffuses to the nucleus, where it acts at mitotic spindles. The degree of metabolism appears to be small, however, as the formation of the metabolite was <10%. The enzyme responsible for the metabolism of tasidotin-C-carboxylate to desprolyl-tasidotin-C-carboxylate remains to be identified.

## CONCLUSION

In summary, no apparent difference between H460- and LOX-implanted mice was observed in the pharmacokinetics of tasidotin or the carboxylate metabolite, suggesting that other mechanisms are responsible for the resistance to tasidotin in the H460 xenograft model. After administration of tasidotin, parent tasidotin concentrations were rapidly cleared from plasma, and concentrations were about equal in plasma and tumors. Tasidotin exposure was not pharmacokinetically linear. Tumor and plasma exposure increased supraproportionally when the dose was increased from 20 to 120 mg/kg. The carboxylate metabolite was cleared from the plasma and tumors much more slowly than tasidotin. Plasma and tumor carboxylate concentrations were less than parent concentrations in those matrices. Carboxylate concentrations in tumors were much larger than in plasma. The carboxylate metabolite contributed 20% to 50% to the total exposure in tumors and was the main moiety responsible for the activity of tasidotin. After administration of tasidotin-C-carboxylate, plasma and tumor tasidotin-C-carboxylate

concentrations were in equilibrium at least initially, but whether tumor tasidotin-C-carboxylate represented intracellular concentrations or extracellular concentrations could not be determined. The true effective-half life of tasidotin-C-carboxylate in mice was on the order of minutes compared with hours after administration of tasidotin. Tasidotin-C-carboxylate showed greater avidity toward tumors than toward plasma, as concentrations were higher in tumors at later time points and the elimination half-life was longer in tumors than in plasma. Tasidotin-C-carboxylate was rapidly metabolized to desprolyl-tasidotin-C-carboxylate, although the amount of desprolyl-tasidotin-C-carboxylate formed appeared small.

## REFERENCES

1. Poncet J. The dolastatins, a family of promising antineoplastic agents. *Curr Pharm Des.* 1999;5:139-162.
2. Supko JG, Lynch TJ, Clark JW, et al. A phase I clinical and pharmacokinetic study of the dolastatin analogue cemadotin administered as a 5-day continuous intravenous infusion. *Cancer Chemother Pharmacol.* 2000;46:319-328.
3. Ray A, Okouneva T, Manna T, et al. Mechanism of action of the microtubule-targeted antimetabolic depsipeptide tasidotin (formerly ILX651) and its major metabolite, tasidotin-C-carboxylate. *Cancer Res.* In press.
4. Ebbinghaus S, Rubin E, Hersch E, et al. A phase I study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously daily for 5 consecutive days every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res.* 2005;11:7807-7816.
5. Cunningham C, Appleman LJ, Kirvan-Visovatti M, et al. Phase I and pharmacokinetic study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously on days 1, 3, and 5 every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res.* 2005;11:7825-7833.
6. Mita AC, Hammond LA, Bonate PL, et al. Phase I and pharmacokinetic study of tasidotin hydrochloride (ILX651), a third-generation dolastatin-15 analogue, administered weekly for three weeks every 28 days in patients with advanced solid tumors. *Clin Cancer Res.* 2006;12:5207-5215.
7. Atack JR, Suman-Chauhan N, Dawson G, Kulagowski JJ. In vitro and in vivo inhibition of prolyl oligopeptidase. *Eur J Pharmacol.* 1991;205:157-163.
8. Boxenbaum H, Battle M. Effective half-life in clinical pharmacology. *J Clin Pharmacol.* 1995;35:763-766.
9. Friedman TC, Orłowski M, Wilk S. Prolyl oligopeptidase: inhibition in vivo by N-benzoyloxycarbonyl-prolyl-proline. *J Neurochem.* 1984;42:237-241.
10. Wilk S. Prolyl oligopeptidase. *Life Sci.* 1983;33:2149-2157.
11. Bausback HH, Ward PE. Vascular, post proline cleaving enzyme: metabolism of vasoactive peptides. *Adv Exp Med Biol.* 1986;198:397-404.